STEREOLOGY – LESSON FROM HISTORY

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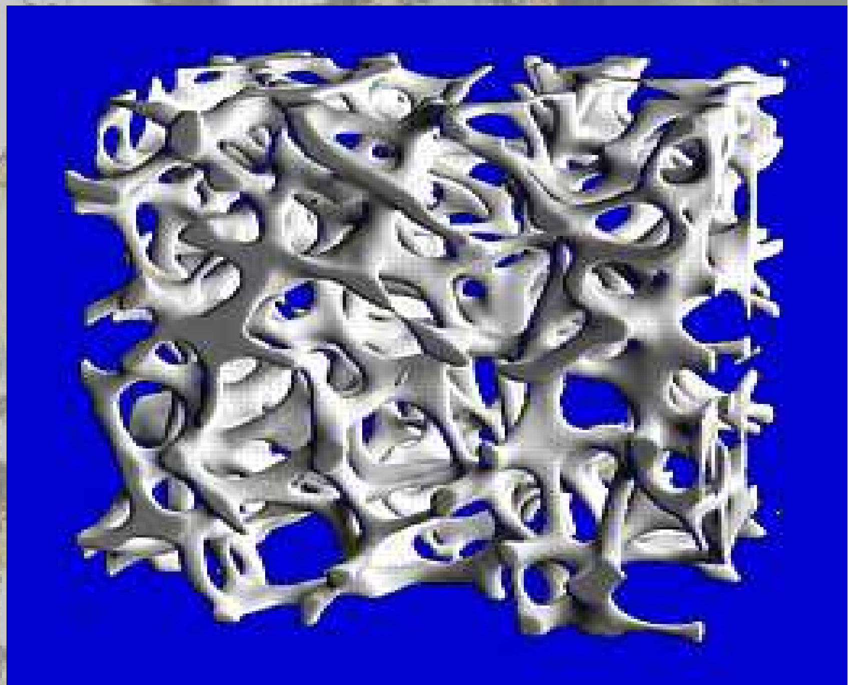
CTU, Prague



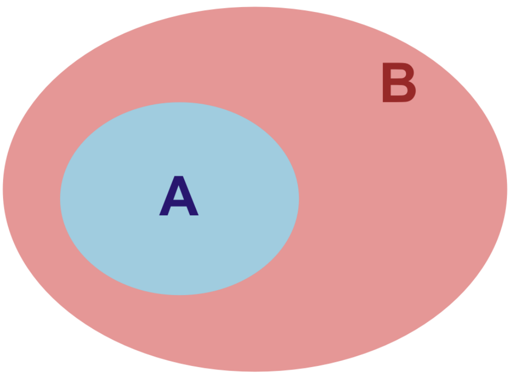
GEOMETRIC PROBABILITY

Classical probability:

– based on combinatorics considerations

– e.g.: probability that two sixes come in two throws:

$$P = \frac{\text{number of cases favourable to the event}}{\text{number of all cases (equally possible)}}$$



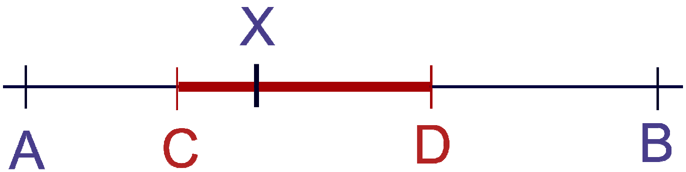
Geometric probability:

– uncountable number of cases

– e.g.: probability that a point lying in the set A lies also in the set B:

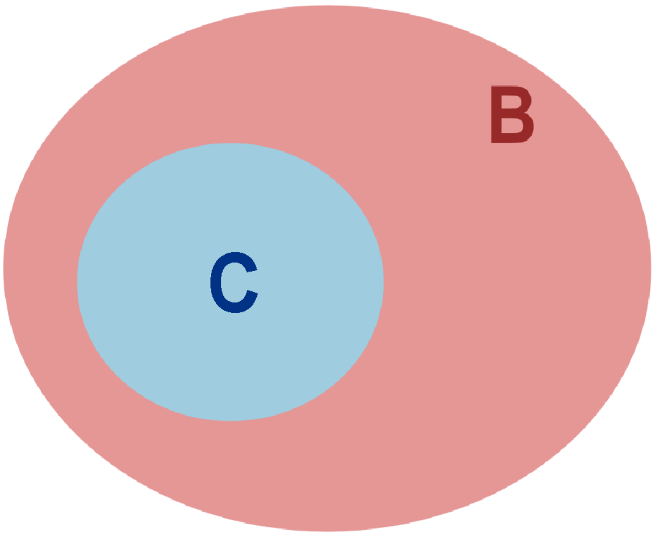
$$P(X \uparrow A \mid X \uparrow B) = \frac{\text{Measure of the set A}}{\text{Measure of the set B}}$$

Points on lines, curves ... measure: length



$$P(X \uparrow CD \mid X \uparrow AB) = \frac{\overline{CD}}{\overline{AB}}$$

Points in regions in plane/space ... measure: area/volume



$$P(X \uparrow C \mid X \uparrow B) = \frac{m(C)}{m(B)}$$

ROOTS OF GEOMETRIC PROBABILITY

Prehistory – isolated problems:

- **Isaac Newton (1642 – 1727), 1664 – 1666**
- **Louis Leclerc, Comte de Buffon (1707 – 1788), 1733, 1777 ... needle and several other problems**
- **Pierre Simon de Laplace (1749 – 1827), 1812**
- **Isaac Todhunter (1820 – 1884), 1857**

History – theory of geometric probability:

- **Since 1865:** **British journal *Mathematical Questions with Their Solutions from the 'Educational Times'*: various problems and exercises concerning GP**

  James Joseph Sylvester (1814 – 1897)

  Morgan William Crofton (1821 – 1895)

  Thomas Archer Hirst (1830 – 1892)

  Arthur Cayley (1821 – 1895) **and others**

- **French mathematicians:**

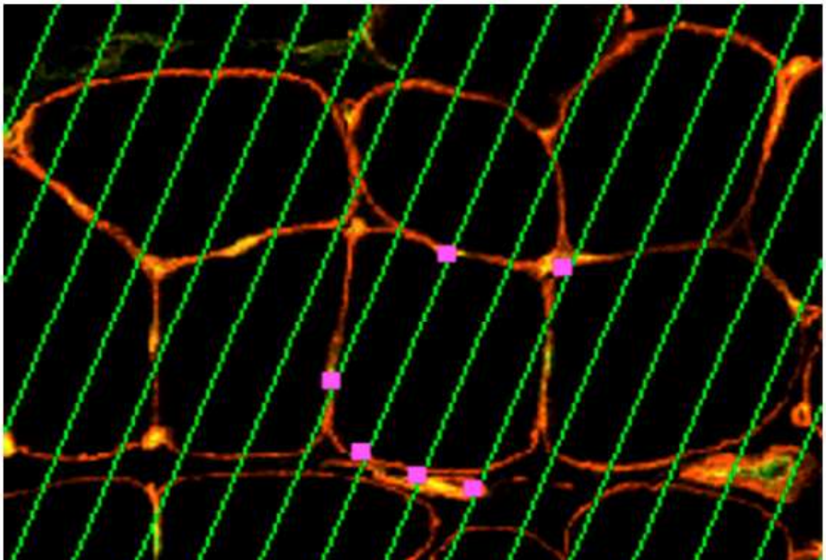
  Gabriel Lamé (1795 – 1870)

  Joseph Bertrand (1822 – 1900)

  Joseph-Émile Barbier (1839 – 1889)

JOSEPH-ÉMILE BARBIER (1839 – 1889)

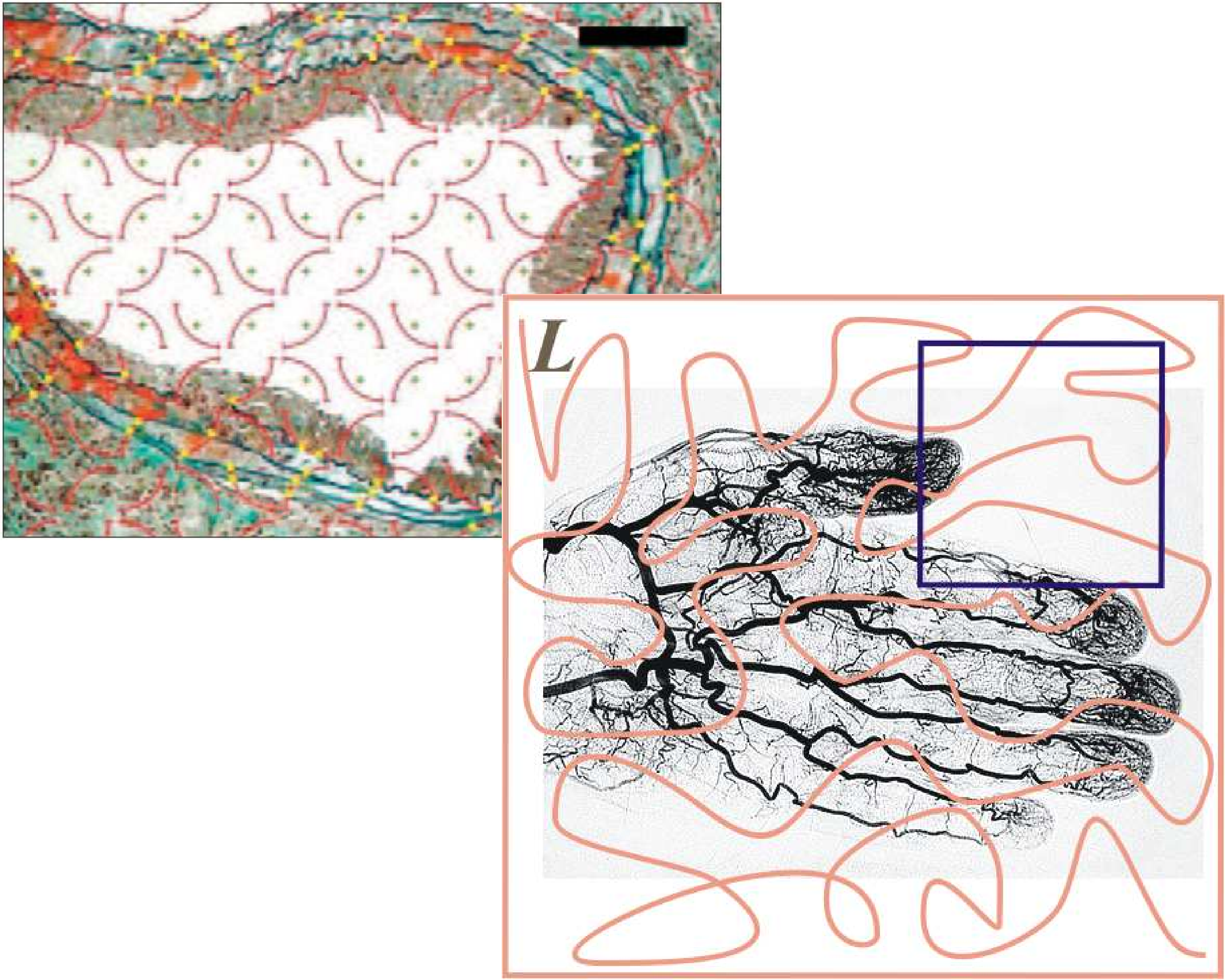
Note sur le problème de l'aiguille et le jeu du joint couvert. **Journal des mathématiques pures et appliquées 5 (1860), 273-286**



$$E(N) = \frac{2}{\pi} \cdot L_A l$$

$$[l] = \frac{\pi}{2} \cdot \frac{1}{L_A} \cdot N$$

$$[l_A] = \frac{\pi}{2} N_L$$

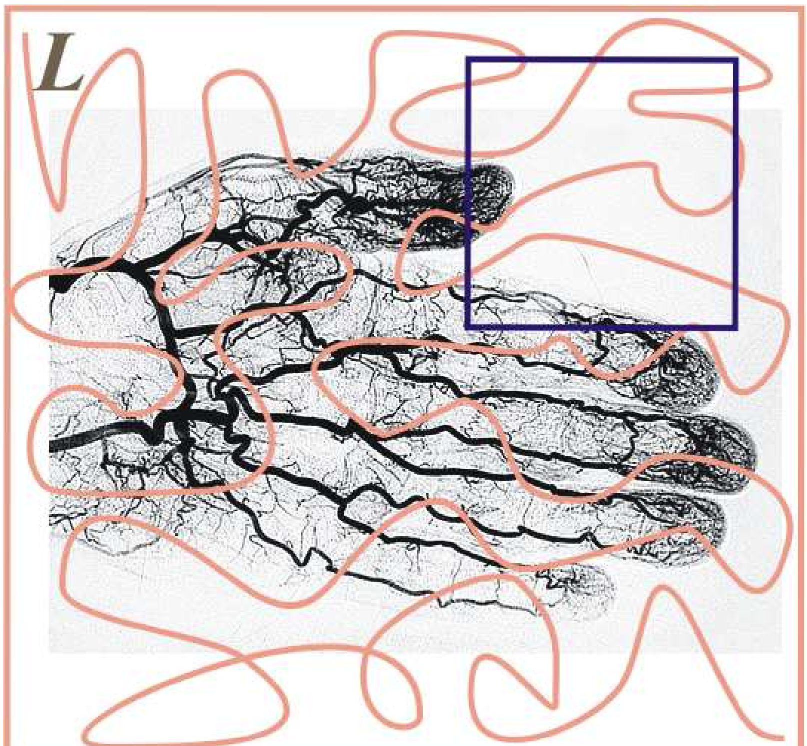

L

Theorem 1: A plane contains a flexible fibre of length L_A meters in each its square metre, taking a variable form, and let another flexible fibre of length l metres be randomly thrown on the plane, then the mean number of intersection points oscillates, independently of the number of trials, around $(2/\pi)\cdot L_A\, l$

$$E(N) = \frac{2}{\pi} \cdot L_A l$$

$$[l] = \frac{\pi}{2} \cdot \frac{1}{L_A} \cdot N$$

$$[l_A] = \frac{\pi}{2} N_L$$



Theorem 2: Let us imagine an unbounded space divided into cubes of one metre edge. Let us suppose that every such cube contains *S* square metres of a cloth (that need not be evolvable into a plane). A fibre of length *L*, which passes randomly through the space, traverses the cloth in (1/2)·S_V *L* points on average.

$$E(N) = \frac{1}{2} \cdot S_V L$$

$$[S_V] = 2 \cdot \frac{N}{L}$$

$$\boxed{[S_V] = 2 N_L}$$

Theorem 3: Each cubic metre of an unbounded space is traversed by a fibre of length L_V metres. Then a cloth of surface area S square metres is intersected by the fibre in $(1/2)\cdot L_V S$ points on average.

$$E(N) = \frac{1}{2} \cdot L_V S$$

$$\boxed{[L_V] = 2N_A}$$

Theorem 4: Suppose finally that each cubic metre of a space contains S_V square metres of cloth. Then the mean length of the intersection of these cloths with another cloth of s square metres is $(3/2)\cdot\pi S_V s$.

$$E(L_P) = \frac{3}{2}\pi S_V s$$

B_A ... length of the intersection in one area unit

After a small correction of the constant:

$$[S_V] = \frac{4}{\pi} B_A$$

EMANUEL CZUBER (1851 – 1925)

Geom. Wahrscheinlichkeiten und Mittelwerte, 1884

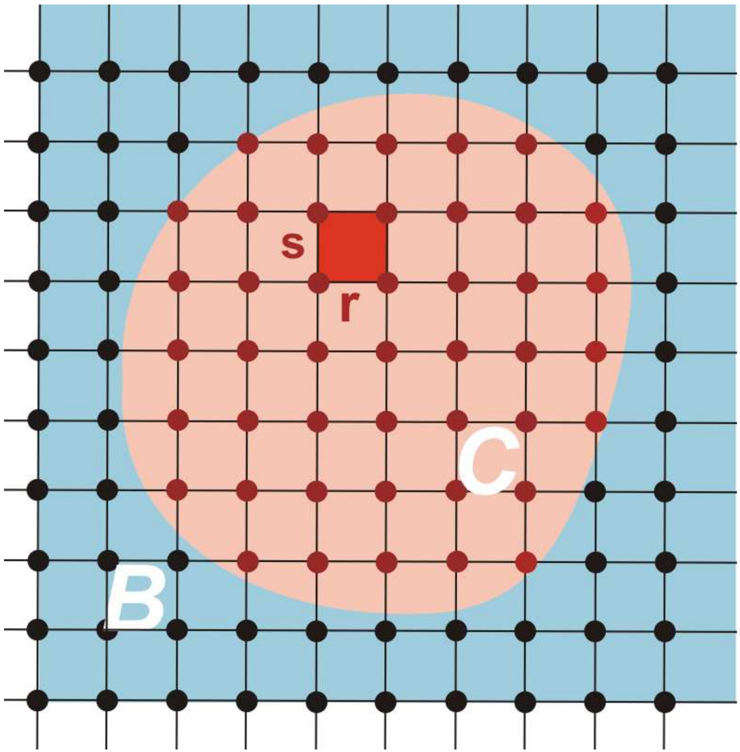
- The first monograph solely devoted to geom. prob.
- Summary of the known theory, detailed theoretical exposition + exercises
- History of the theory (Buffon up to „present")
- Explicit citation of French and English predecessors
- New ideas and generalizations

e.g., Crofton (1868) derived key theorems concerning sets of points and straight lines in a plane & briefly outlined possible generalization to 3D;

this was done in full details by Czuber

Points in the plane – area estimation

$$P(X \uparrow C | X \uparrow B) = \frac{A(C)}{A(B)} = \overline{\left(\frac{N_{hits}}{N_{total}}\right)}$$



$$A(C) = A(B) \cdot \overline{\left(\frac{N_{hits}}{N_{total}}\right)}$$

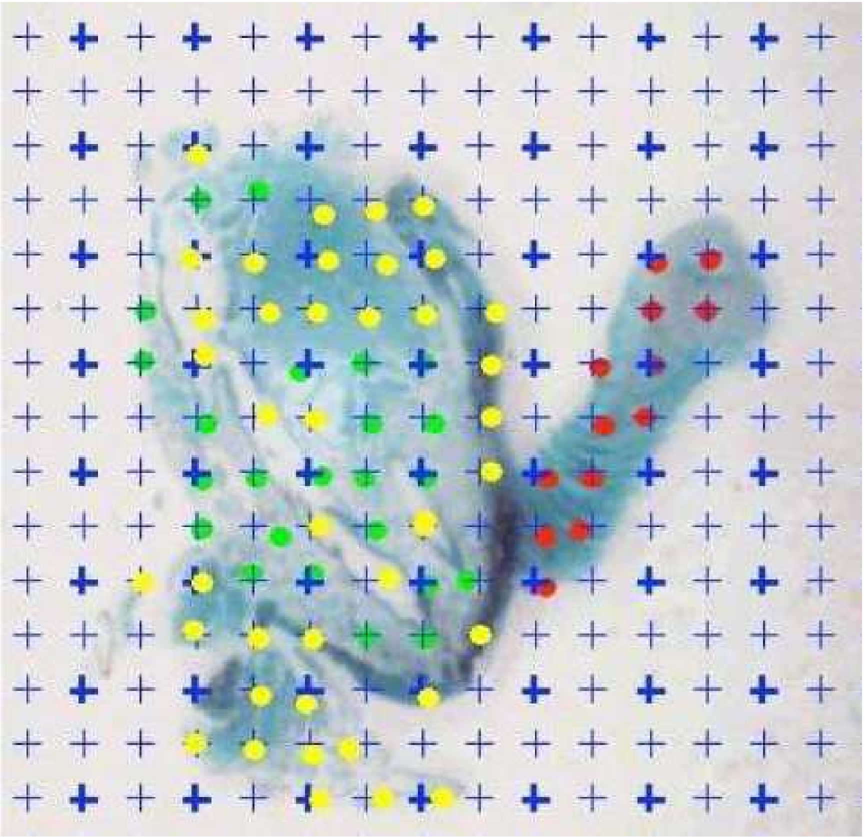
Estimation:

$$A(C) = \frac{A(B)}{N_{total}} \cdot \overline{N_{hits}}$$

$$\boxed{A(C) = rs \cdot \overline{N_{hits}}}$$

Points in the plane – area estimation

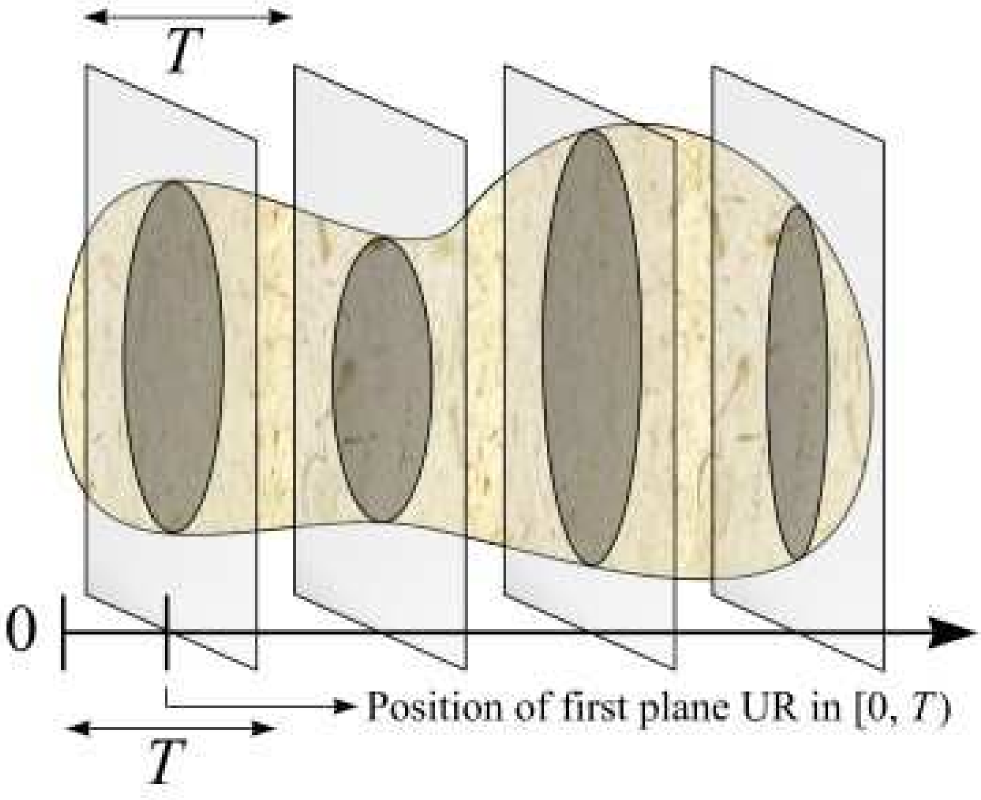
$$P(X \uparrow C | X \uparrow B) = \frac{A(C)}{A(B)} = \overline{\left(\frac{N_{hits}}{N_{total}} \right)}$$



$$\boxed{A(C) = rs \cdot \overline{N_{hits}}}$$

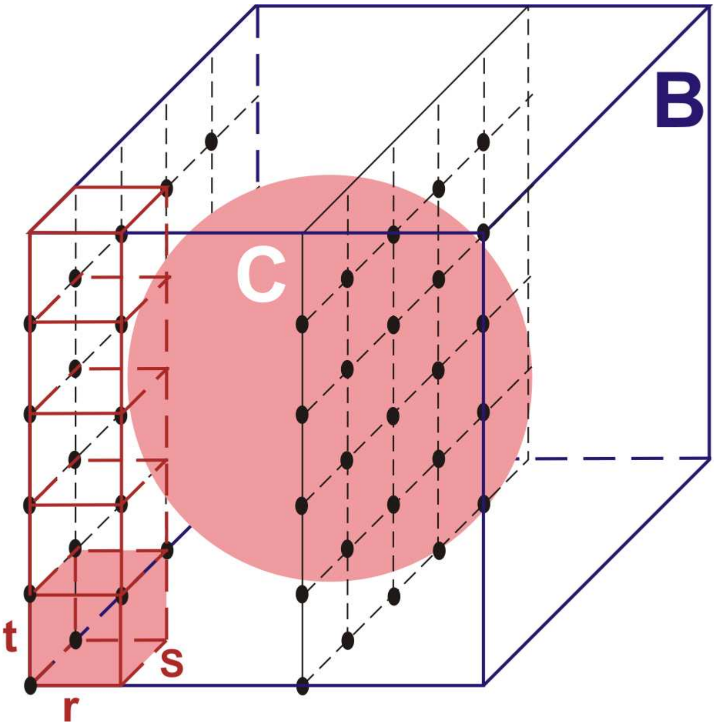
$$A_A = P_P$$

Points in the space – volume estimation



Points in the space – volume estimation

$$P(X \uparrow C | X \uparrow B) = \frac{V(C)}{V(B)} = \overline{\left(\frac{N_{hits}}{N_{total}}\right)}$$



$$V(C) = V(B) \cdot \overline{\left(\frac{N_{hits}}{N_{total}}\right)}$$

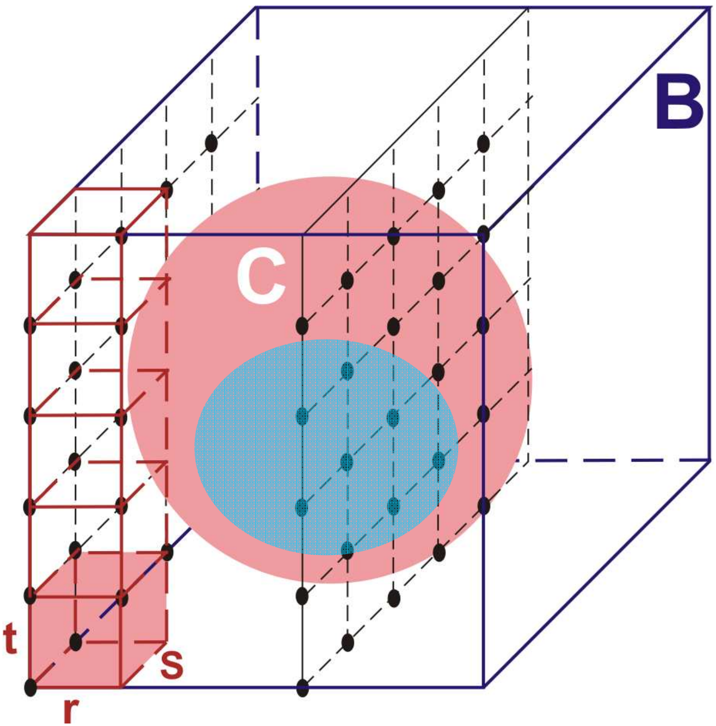
Estimation:

$$V(C) = \frac{V(B)}{N_{total}} \cdot \overline{N_{hits}}$$

$$\boxed{V(C) = rst \cdot \overline{N_{hits}}}$$

Points in the space – volume estimation

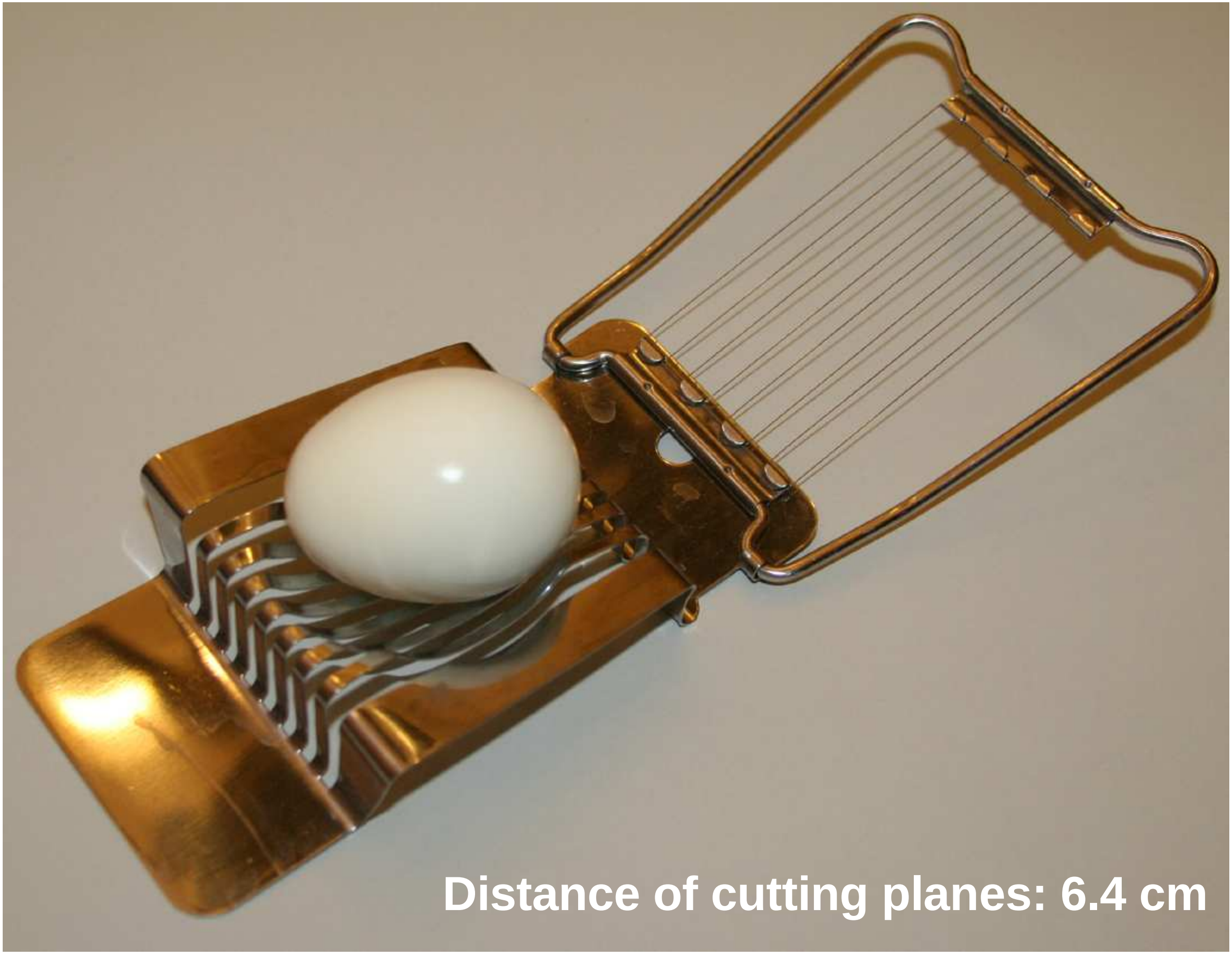
$$P(X \uparrow C | X \uparrow B) = \frac{V(C)}{V(B)} = \overline{\left(\frac{N_{hits}}{N_{total}}\right)}$$

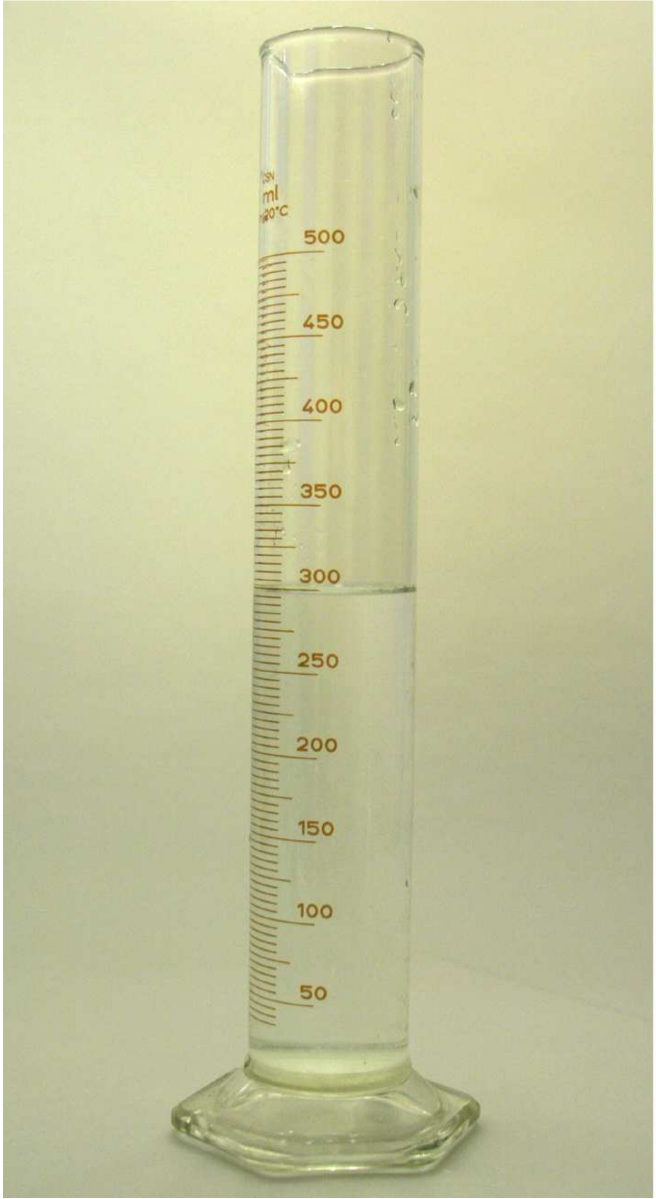


$$\boxed{V(C) = rst \cdot \overline{N_{hits}}}$$

$$V_V = A_A = P_P$$

Example: Estimation of the volume of an egg:

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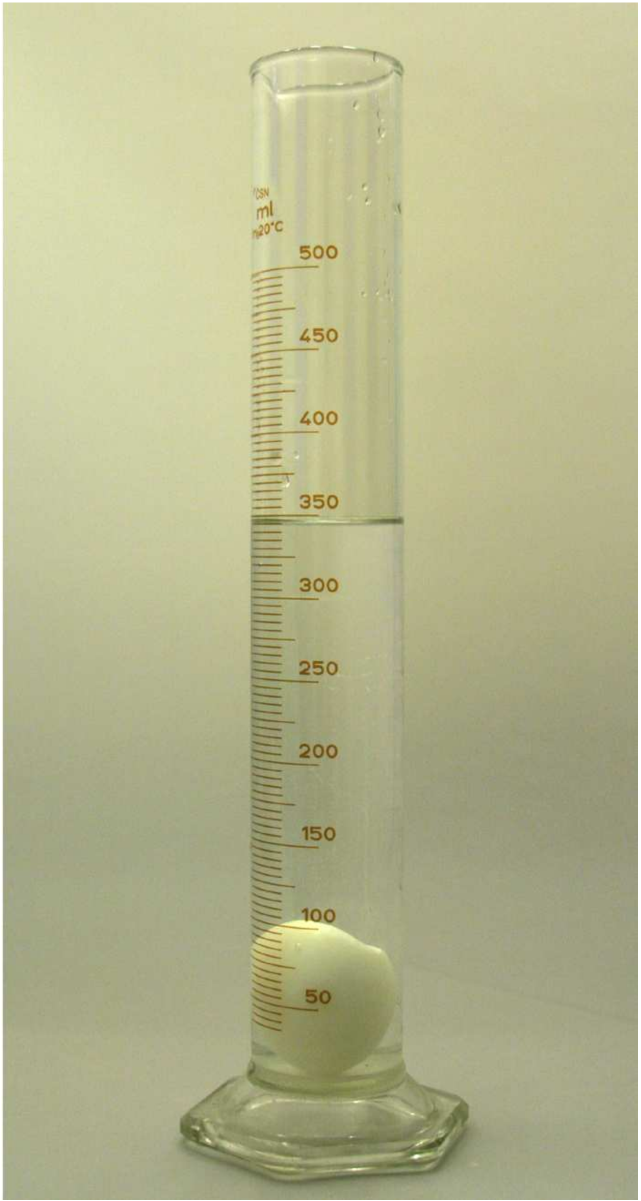
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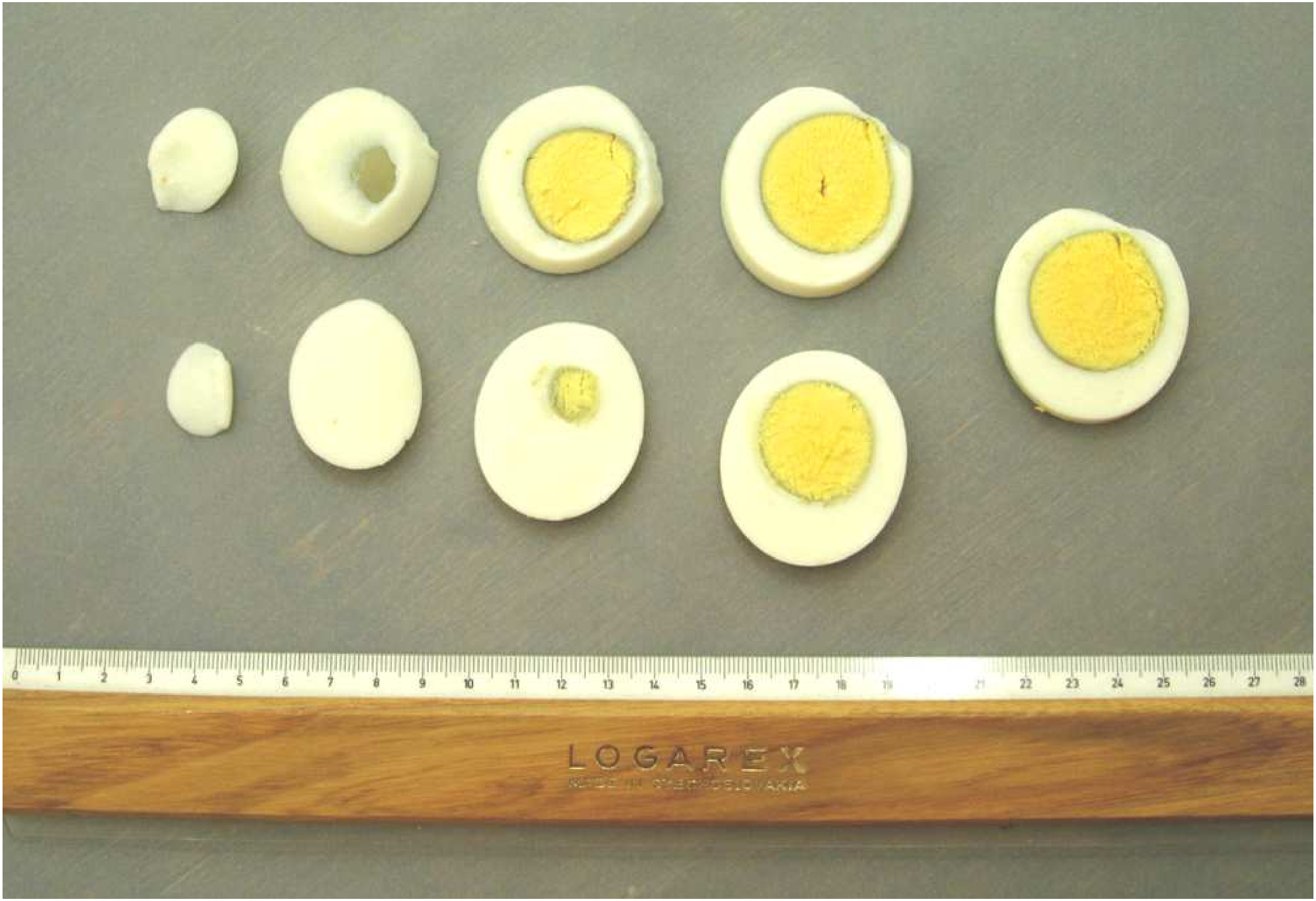
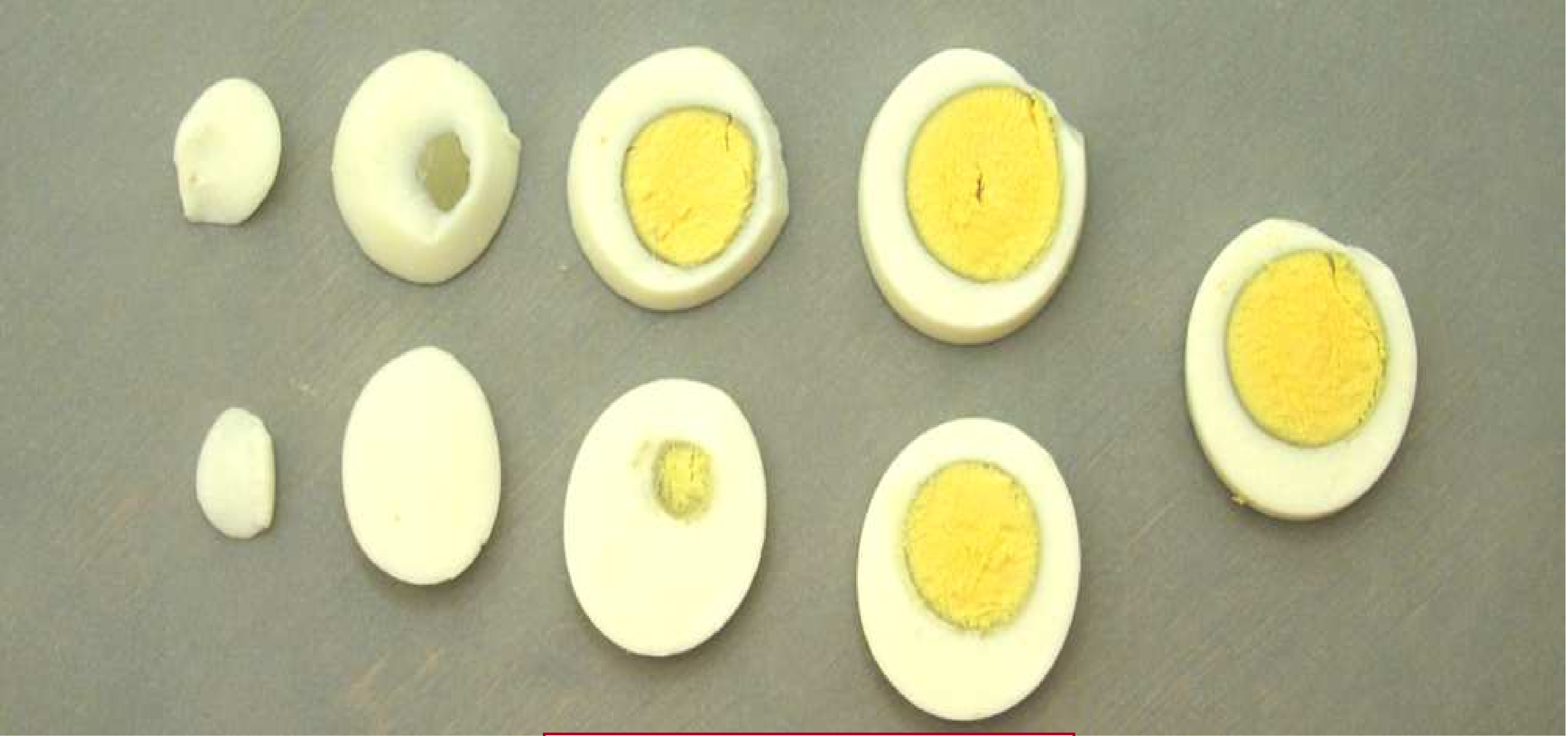

LOGAREX

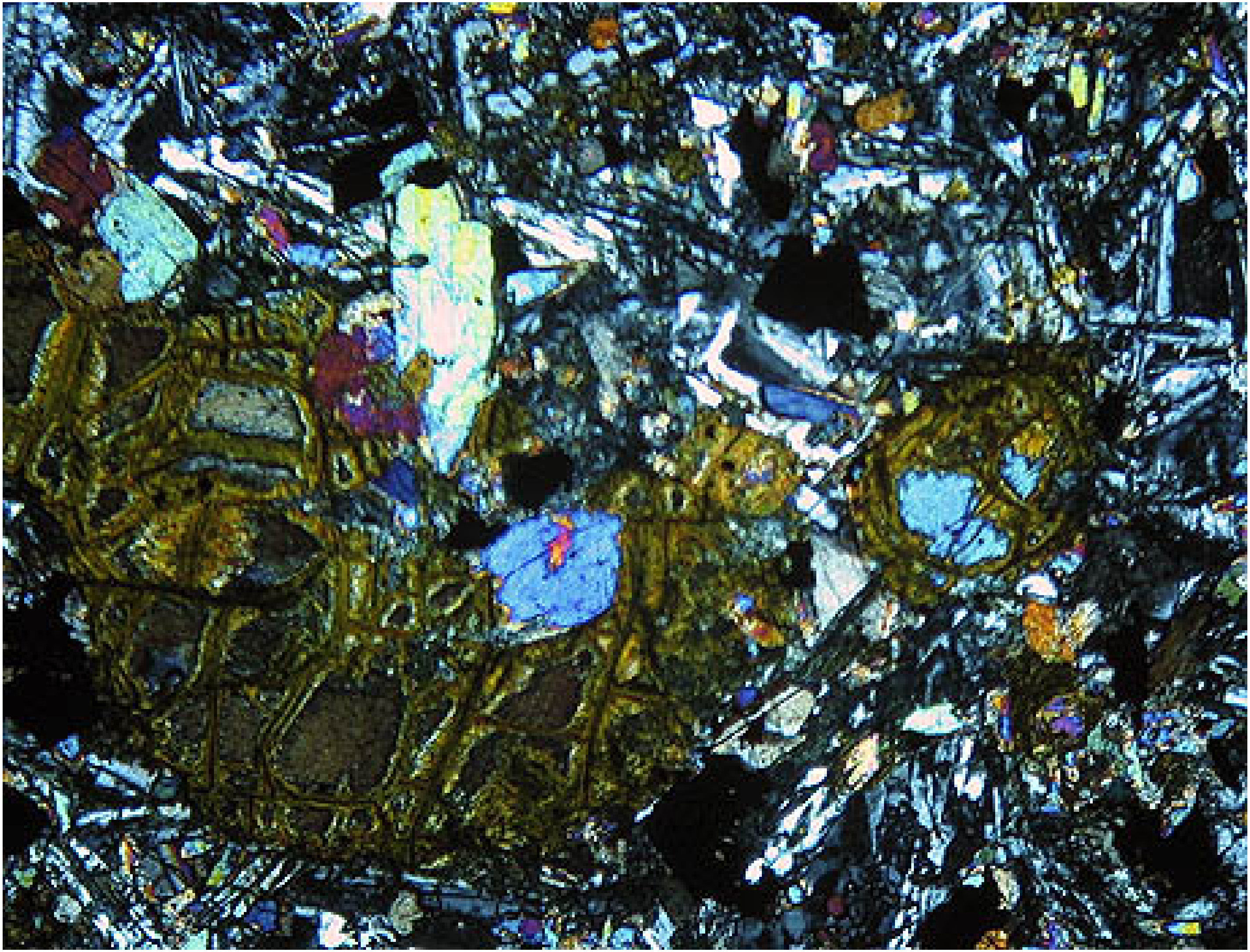


$$V_V = A_A = P_P$$

V_V ... average fraction of volume of the egg occupied by yolk

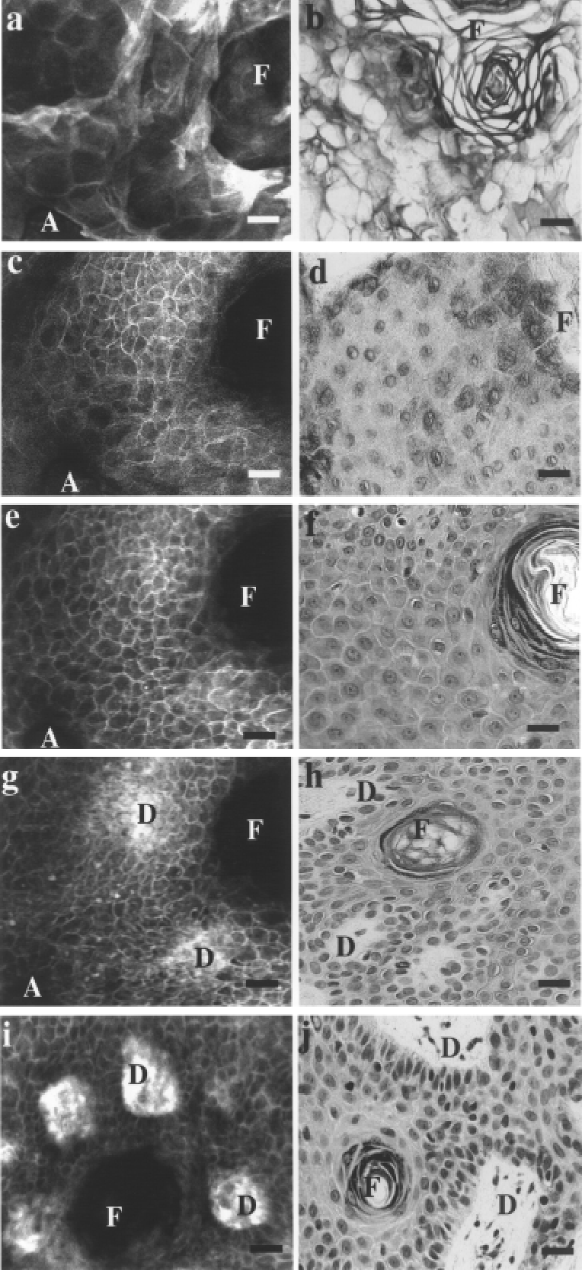
A_A ... average fraction of area on a plane section occupied by yolk

P_P ... average proportion of test points covered by yolk



Dolerite

Sandstone



Important practical implementation of the spatial grid: in confocal and transmission microscopy

cutting thin section of a tissue

↳ visualization of an optical section (an image of the focal plane only) situated inside a <u>thick</u> section

epidermis – confocal sections

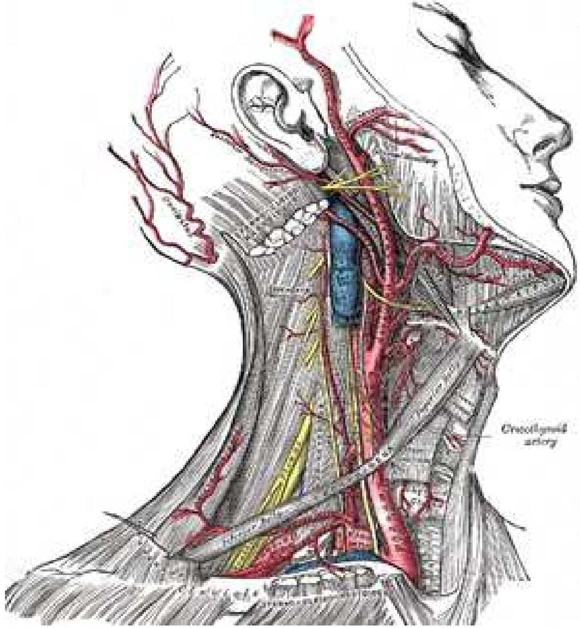
Population

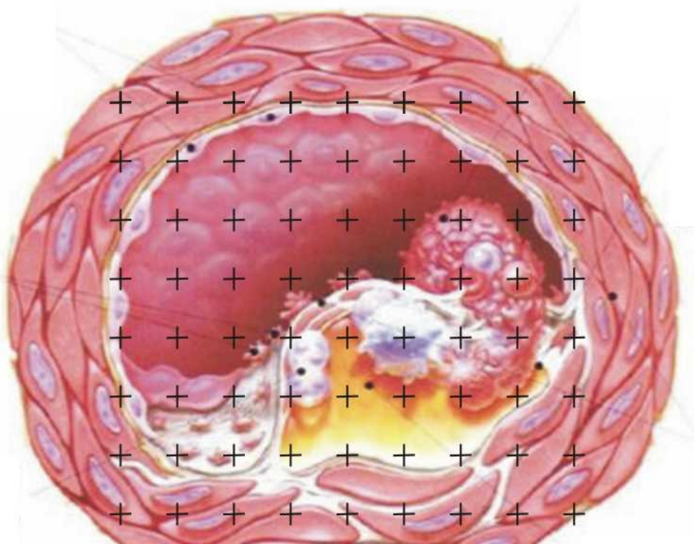
Random sample

Statistics

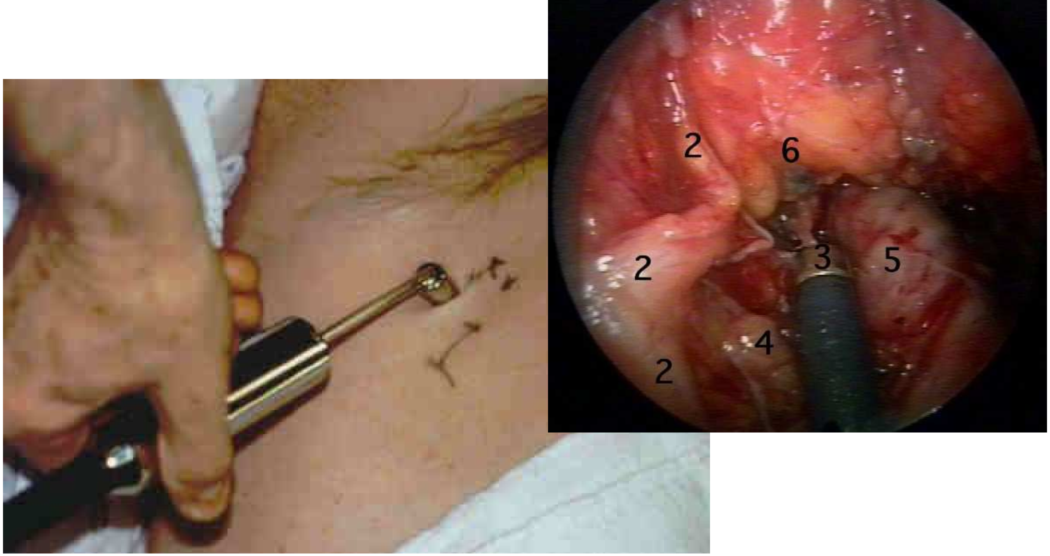
Stereology

A body of math. methods for the estimation of certain geometric characteristics of three-dimensional structures on the basis of probes of a lower dimension (plane sections, linear projections).





Tissue probe



Geological exploration

Oil exploration/well

ACHILLE ERNEST OSCAR JOSEPH DELESSE
(1817 – 1881)

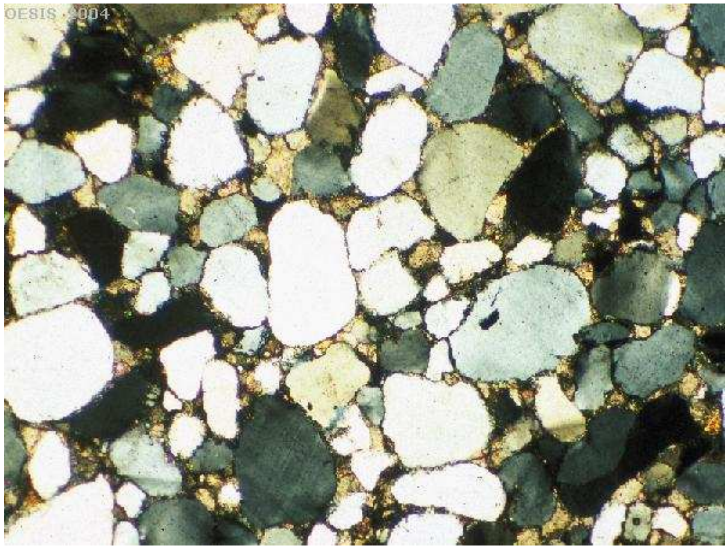
French geologist and mineralogist

***Procede machanique pour determiner la composition des roches*, C.R. Acad. Sci. 25 (1847), p. 544**

Quantitative image analysis

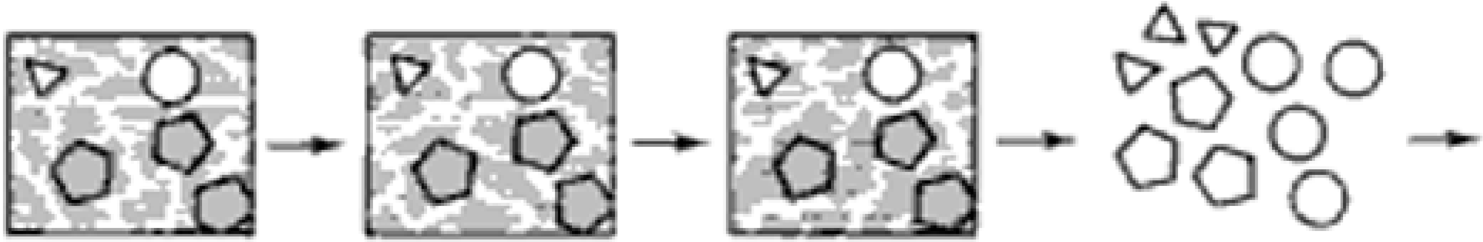
Area density of the investigated mineral in the section

= volume density in the rock



Assumptions:

- **random section**
- **section area >> grains**
- **enough large section (statistically sufficient amount of grains)**
- **the rock is spatially homogenous**



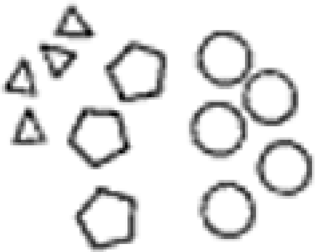
polished

covered with waxed transp. paper, exposed portions of the mineral traced

glued on tin foil (known weight)

traces of the minerals cut out

Volume fraction of a mineral Y:



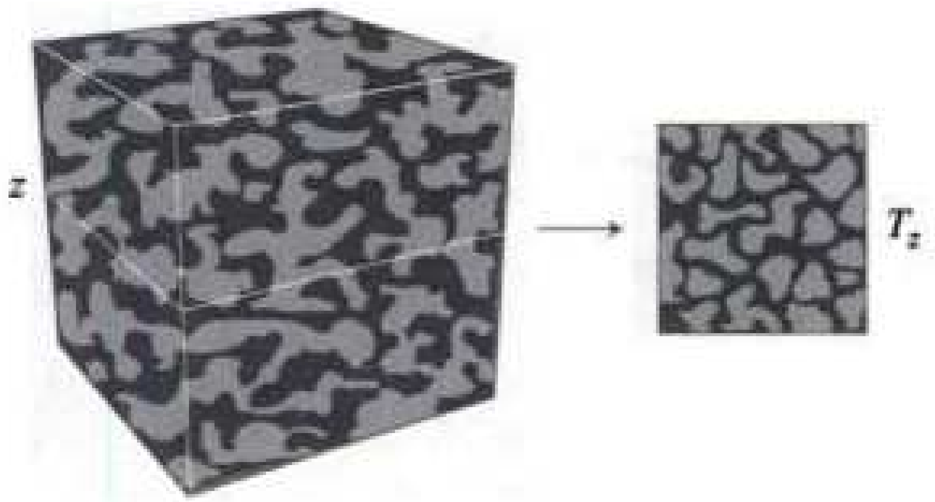
ordering

weighing of groups

$$\frac{A_Y}{A_T} = \frac{V_Y}{V_T}$$

$$V_V = A_A$$

Unit cube of a rock *X*



Y **... investigated mineral of the volume *V*(*Y*)**

T_z **... horizontal plane in the height *z***

Fubini: solid volume equals the integral of areas of planar sections

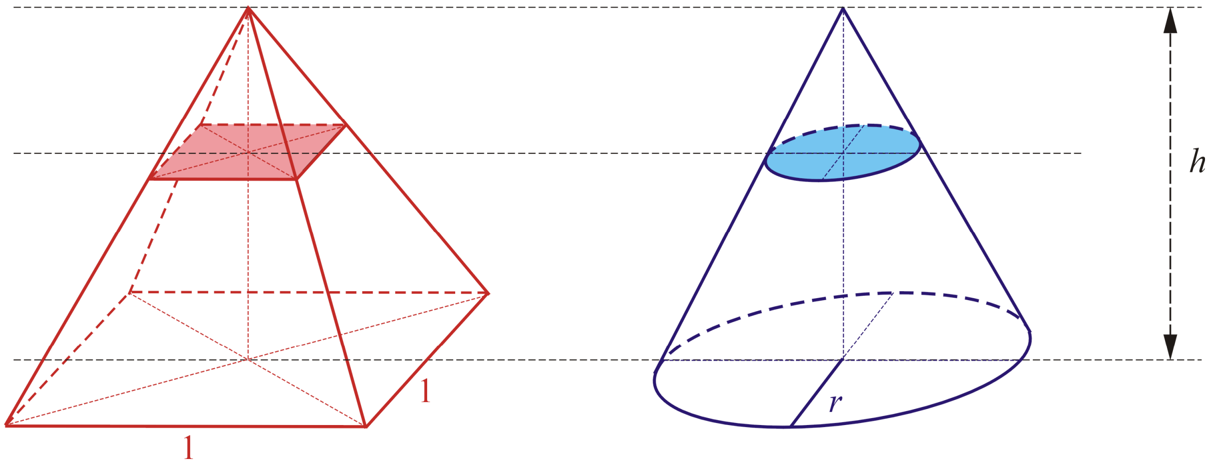
$$\int A(Y \cap T_z)dz = V(Y)$$

$$\rightarrow \text{expectations} \rightarrow A_A = V_V$$

3-dimensional reconstruction of an object is not necessary for volume estimation

Cavalieri’s principle:

If two solid objects have the same height and the ratio of areas of cross-sections on planes parallel to the base in the same distance from it is constant, then the ratio of volumes of these solid objects is the same.



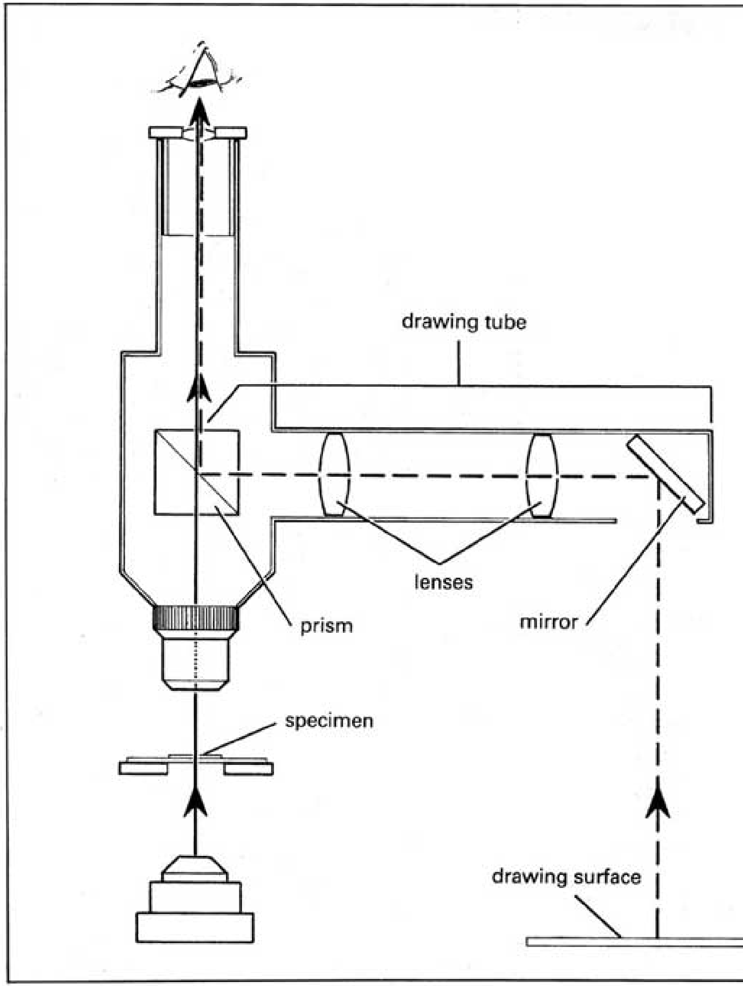
The ratio of volumes of the investigated component and the whole sample can be estimated from the ratio of section areas – provided the conditions for a proper statistical selection are satisfied.

HENRI CLIFTON SORBY

British geologist & anthropologist

1856: ***On slaty cleavage as exhibited in the Devonian limestones of Devonshire*, Phil. Mag., v. 4, no. 11, p. 20–37.**

Camera lucida (Delesse method)



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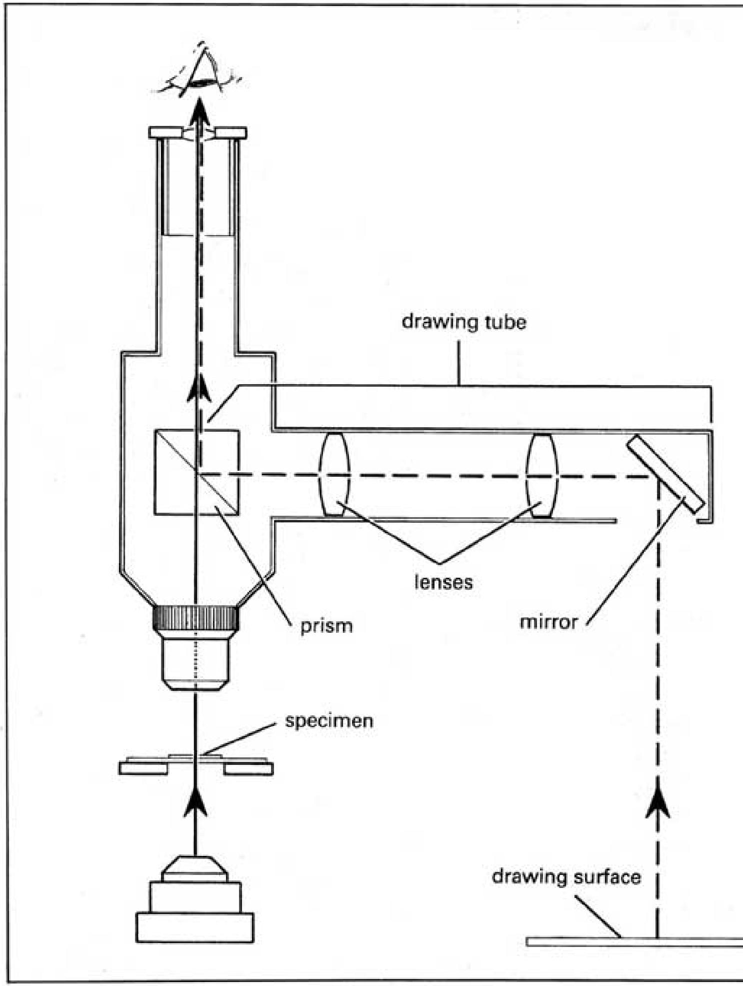
Camera lucida (Delesse method)

A. Johannsen:

***A planimeter method for the determination of the percentage composition of rocks*. Jour. Geology, 27 (1919), 276–285**

Camera lucida

areas determined by a platimeter



J. Joly: *The petrological examination of Paving-Sets*. Proc. Roy. Dublin Soc. 10 (1903), p. 62–92.

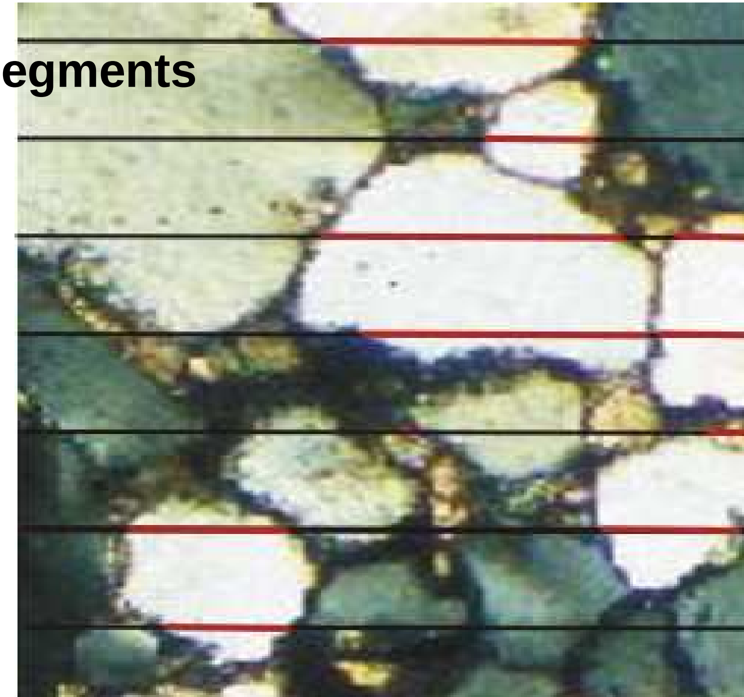
cutting and weighting $\rightarrow$ graphical summing

AUGUST KARL ROSIWAL (1860 – 1913)

Austrian geologist, mineralogist, petrographer

***Ueber geometrische Gesteinsanalysen. Ein einfacher Weg zur ziffremassigen Foxtstellung des Quantitätsverhäitnissos der Mineralbestandtheile gemengter Geneine*. Verhandlungen der k.k. Geolog. Reichsanstalt Wien, 5(6) 143–174.**

Instead of regions: length of line segments



linear fraction of the mineral Y

= area fraction of *Y*

= volume fraction of *Y*:

$$\frac{L_Y}{L_T} = \frac{A_Y}{A_T} = \frac{V_Y}{V_T}$$

William R. Thompson

***Quantitative Microscopic Analysis*, Journal of Geology 38(1930), 193**

Andrej Alexandrovič Glagolev (1894 – 1969)

***On the geometrical methods of quantitative mineralogic analysis of rocks.* [russisch], Trans. Inst. Econ. Min., Moskva, 59 (1933), 1–47**

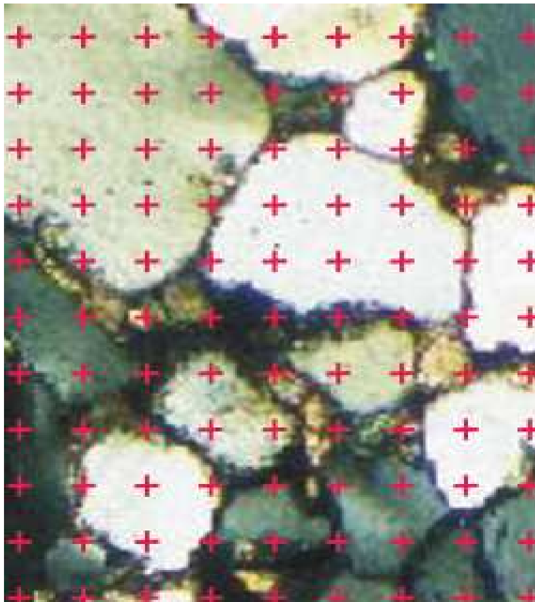
***Quantitative analysis with the microscope by the point method.* Engineering and Mining Journal 135(1934), p. 399**

Regular point grid randomly put on the plane section

Number of points that hit the investigated component

$$V_V = A_A = P_P = L_L$$

generalization of Newton's circle and Buffon's carreau



Stereology & biomedicine

First-order morphological parameters:
volume, surface area, length, number

Essentially every field of biomedical research at one time or another focuses on changes in one of the four first-order parameter

– degeneration, toxicity, atrophy/hypertrophy, dysgenesis, proliferation

H. W. Chalkey, 1943:
***Method for quantitative morphological analysis of tissues*. Jour. Natl. Cancer Inst. 4, 47-53**

one of the first to apply the point counting method to histological images

The bulk of biological and medical knowledge:

QUALITATIVE OPINIONS OF EXPERTS

Limitations:

expert knowledge: highly focused on a particular tissue

⇒ almost as many experts as tissues in the body

e.g., liver biopsies of alcoholics, brain degeneration in Alzheimer's disease

poor reproducibility between observers

1960's: SEMIQUANTITATIVE TERMS for differentiating morphological changes (e.g., none, mild, moderate, severe)

Problems: data are not amenable to powerful methods of statistical analysis;

one must rely on less rigorous nonparametric approaches to test whether inferences are likely to be true on the basis of probability

1961, Feldberg, Black Forest, Germany;
Prof. Hans Elias, biologist:

meeting of diverse researchers from fields of biology, geology, engineering and materials sciences

Aim: to benefit scientists in several disciplines who had one thing in common: struggling with the quantitative analysis of 3-D images based on their appearance on 2-D sections.

At this meeting, Prof. Elias suggested "stereology" as a useful term to describe their discussions.

→ Prof. Elias sent a small announcement on the proceedings to the journal Science

→ large response from researchers in academia, government agencies, and private industry at institutions around the world

1962: **the International Society for Stereology (ISS) was established with the 1st Congress of the International Society for Stereology in Vienna, Austria. Elias elected the founding president**

→ every other year

⇒ biologists discovered that their stereology colleagues in different fields had developed practical approaches that would be of immediate use in their research

1960's: technological innovations in microscopy

⇒ biologists could view tissues, cells, blood vessels and other objects in tissue with greater clarity and specificity than ever before

Developments:

- **the availability of affordable, high-resolution optics for light microscopy**
- **refinements in electron-microscopy instruments and methods for preparation of specimens**
- **immune-based visualization of specific proteins in biological tissue (immunocytochemistry)**

still rather laborious methods

e.g., in the 1960s, for example, a worker in one influential publication spent two years counting 242,681 cells in a particular area on one side of the brain

1970's: important breakthrough:

mathematicians joined the ISS and began to apply their unique expertise and perspective to problems

- **biological objects cannot be modeled as classical shapes (spheres, cubes, straight lines, etc.), Euclidean geometry formulas do not apply**
- **rejected so-called 'correction factors' intended to force biological objects into Euclidean models based on false and non-verifiable assumptions**
- **the correct foundation for quantification of arbitrary, non-classically shaped biological objects: stochastic geometry and probability theory**
- **developed efficient, unbiased sampling strategies for analysis of biological tissue**
- **Do More, Less Well (Prof. Ewald Weibel)**

Sampling theory $\Rightarrow$ accurate estimates of changes within a population by making a relatively small number of measurements in a few randomly sampled individuals

Ewald Weibel, 1972:

Structure and function are strictly interdependent

Example: structure and function of the lung

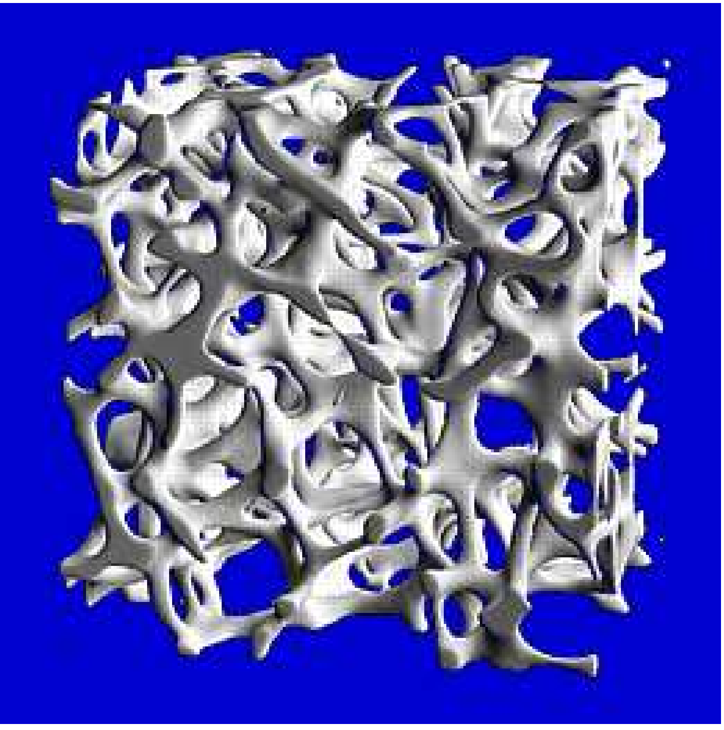
- **calculating diffusion capacity from physiological information on oxygen uptake and from stereological information**

  $\rightarrow$ **estimate of the limit of performance of the pulmonary gas-exchange apparatus under ideal or optimal conditions**

= the first example of the usefulness of stereology in biology and medicine

OSTEOPOROSIS

Half of women over seventy will have a fracture as a result of osteoporosis. For many of them, this will lead to a decline in their quality of life and independence.



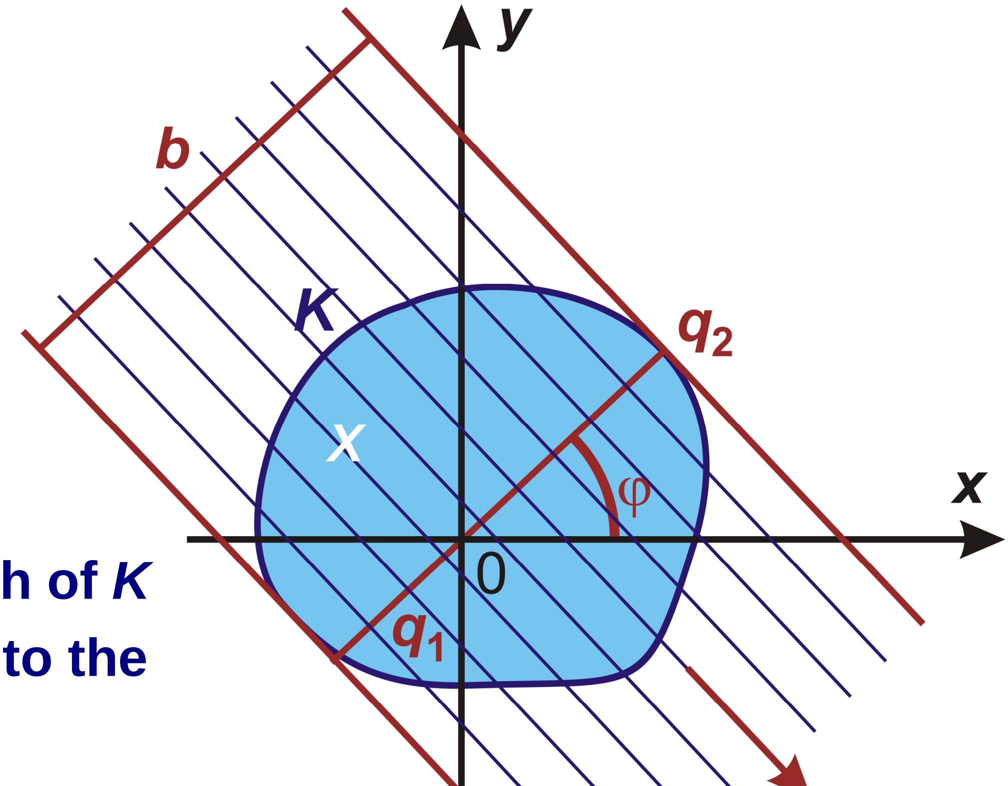
- **bone loss**
- **significant changes in bone micro-architecture, tissue composition and micro-damage**
- **Celluls processes and molecular signalling pathways governing pathological bone resorption have been identified to a certain extent.**

Morgan William Crofton, 1868

Curves in the plane and interactions with lines

Theorem: Measure of lines hitting a closed convex curve in the plane is equal to the length of the curve.

$$\int_0^\pi \mathbf{b}\,\mathbf{d}\varphi = \mathbf{L}$$

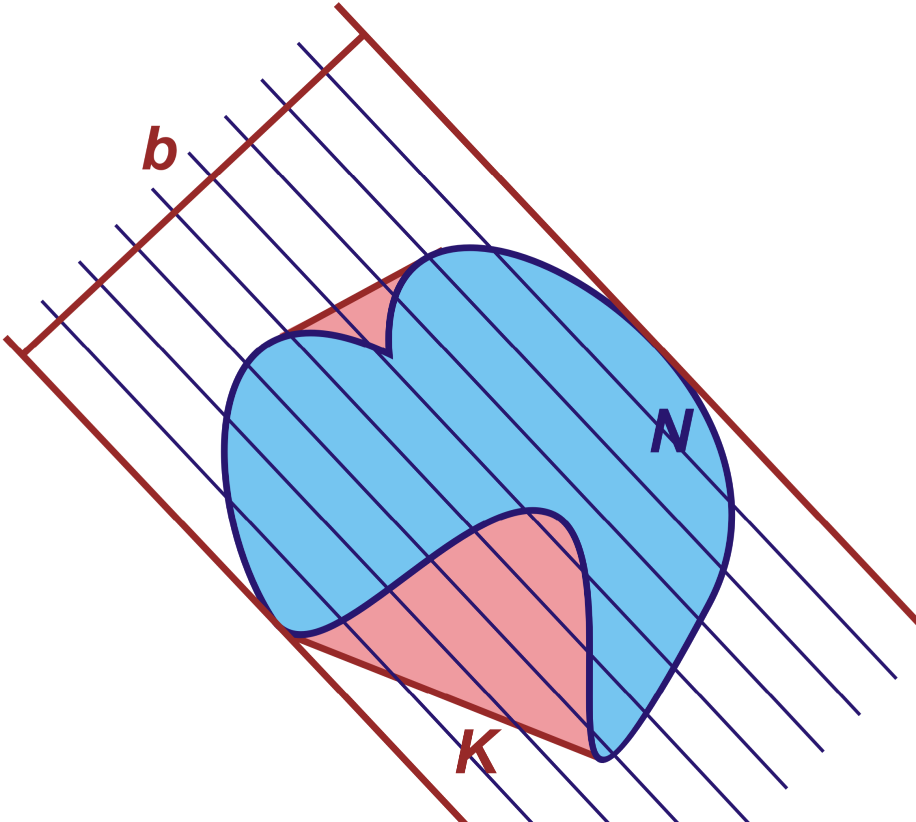


b **... projection length of *K* (convex region *X*) into the given direction**

Curves in the plane and interactions with lines

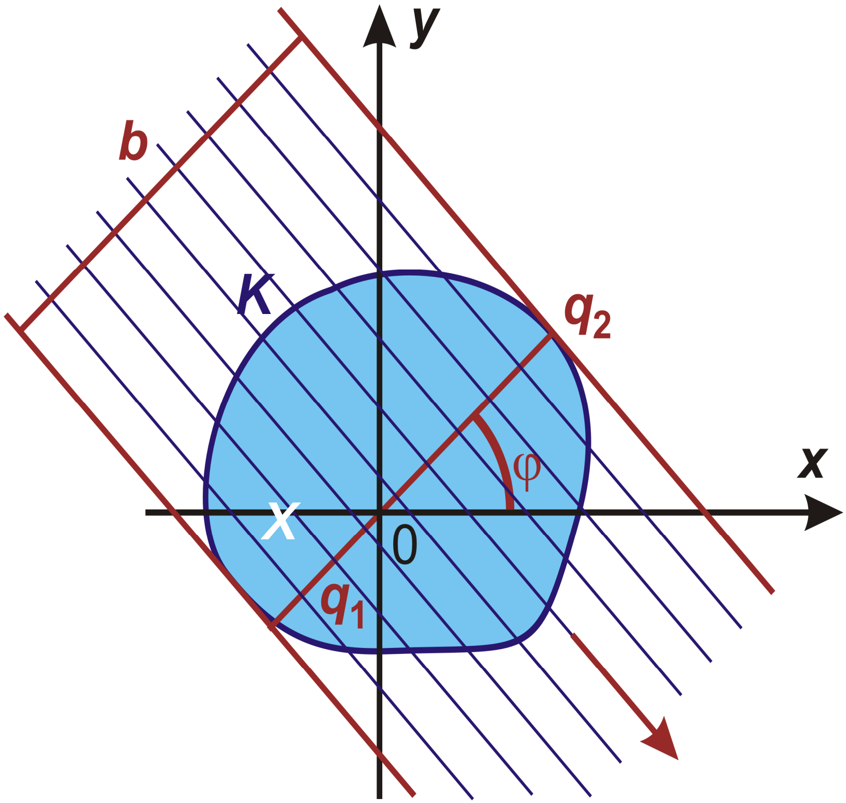
Theorem: Measure of lines hitting a closed nonconvex curve in the plane is equal to the length of the fibre tightly taut around the curve.

$$\int_0^{\pi} b\, d\varphi = L$$



Theorem: Measure of lines hitting a closed convex curve in the plane is equal to the length of the curve.

$$\int_0^\pi b d\varphi = L$$



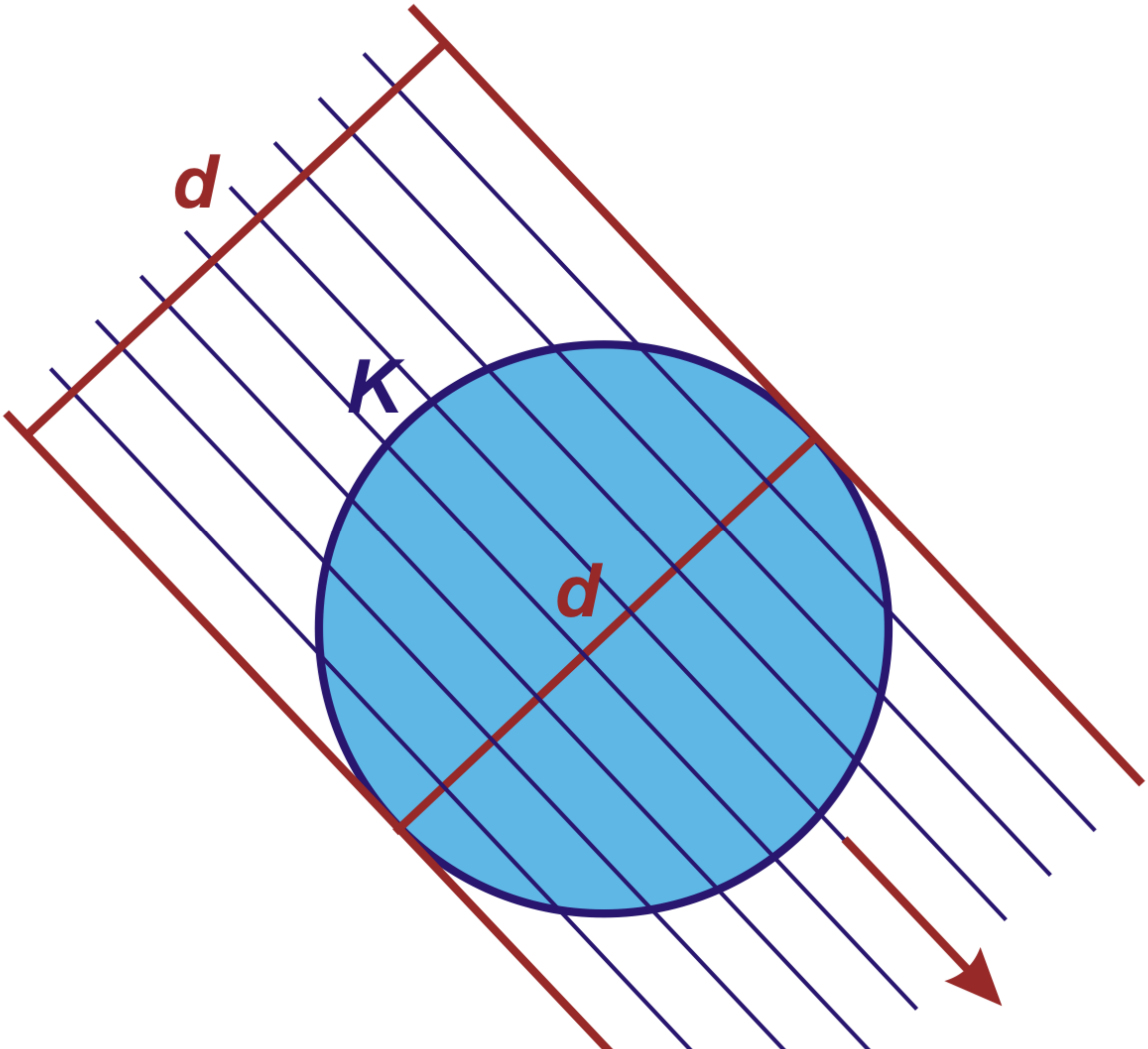
mean projection length of *X* into the isotropic bundle of directions:

$$w = \frac{1}{\pi}\int_0^\pi b d\varphi$$

$$\boxed{L = \pi w}$$

Crofton-Cauchy Formula

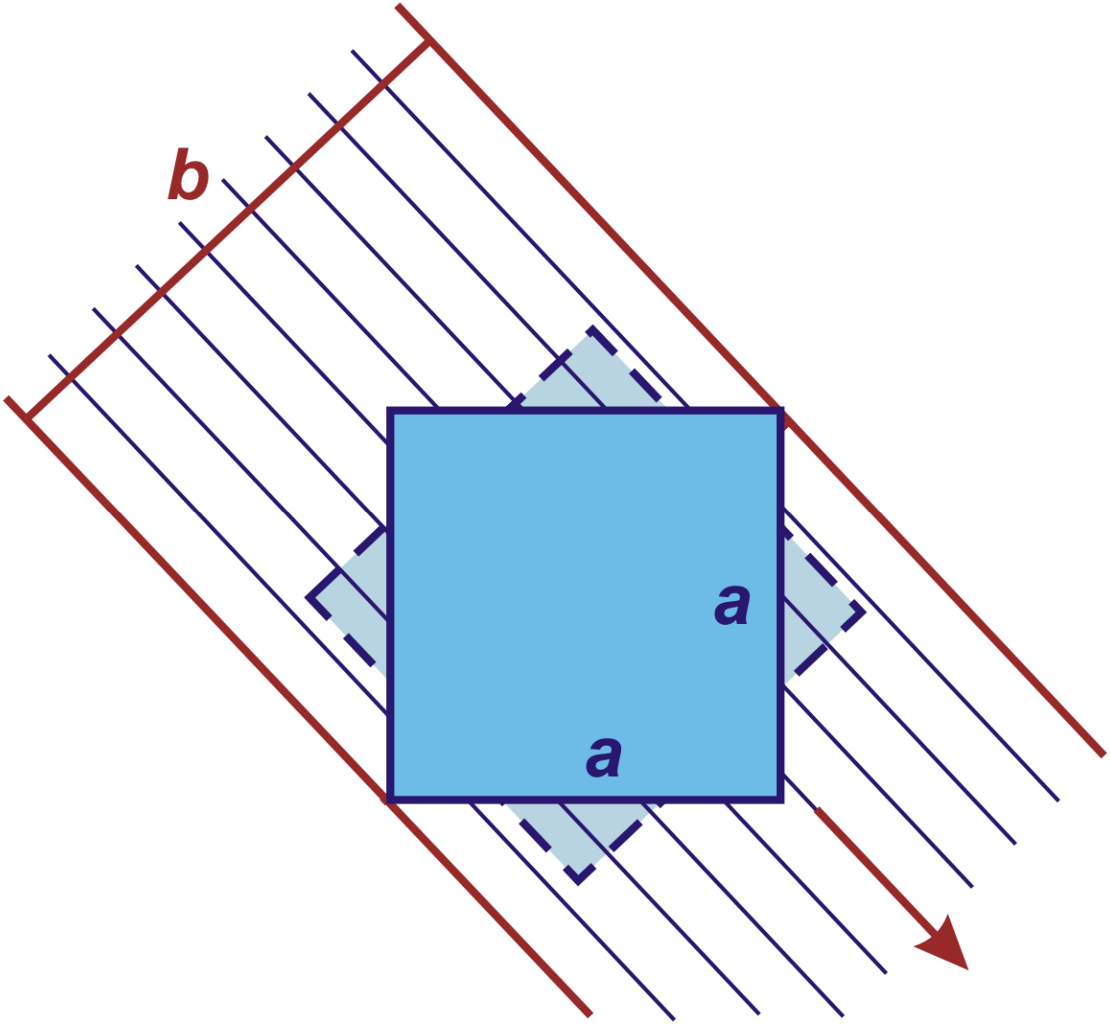
Example: circle



$w = d$

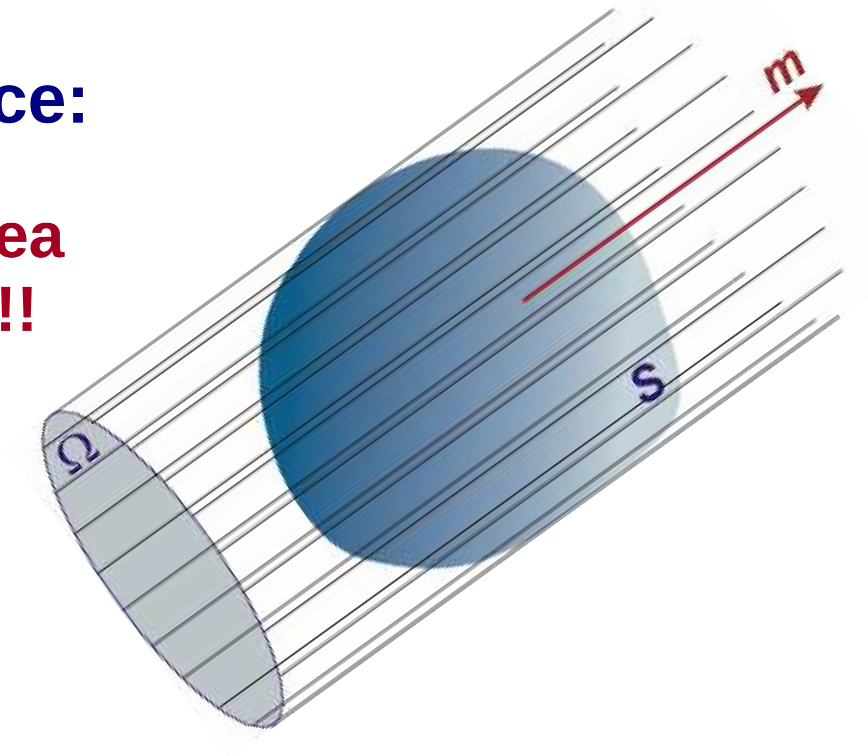
$L = \pi d$

Example: square



$w = 4a/\pi$

Analogously in space:



4∗mean projection area
= surface in general !!!

sphere: $S = 4{*}\pi R^{2}$

cube: $6a^{2}/4$ = mean projection area

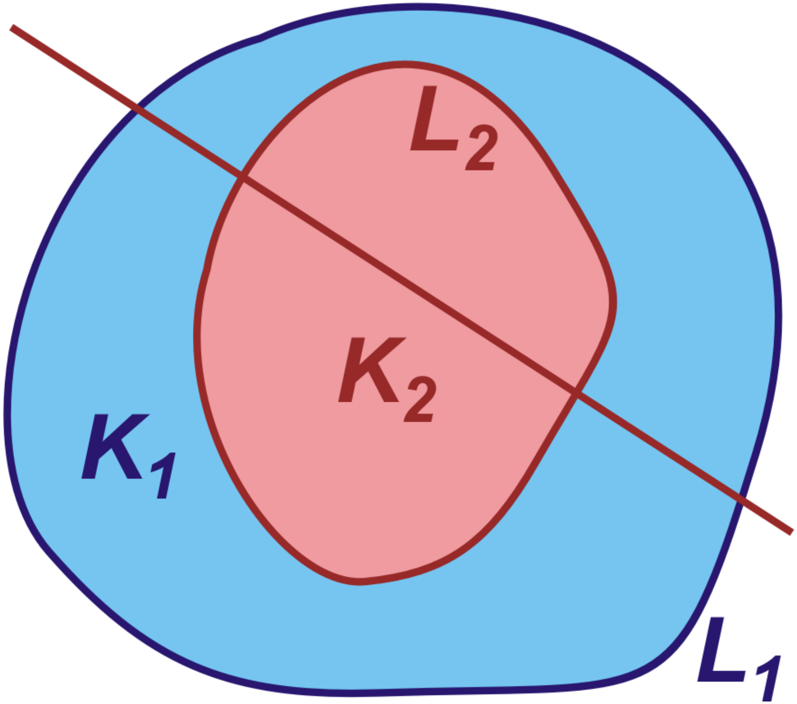
<u>Convex bodies</u>:

proportionality between mean projection measures of an object and its boundary measures !!!

Theorem:

Probability p that a line hitting a bounded convex region K_1 with the perimeter L_1 hits also another convex region K_2 lying inside K_1 and having the perimeter L_2:

$$p = \frac{L_2}{L_1}.$$

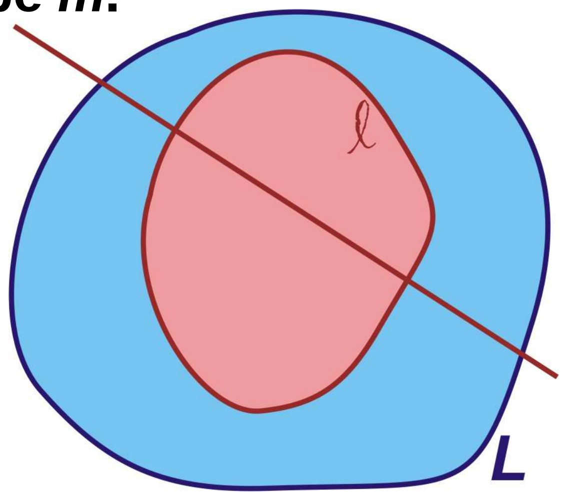


Emanuel Czuber, 1884

Remark: on this result an experimental rectification of a closed convex curve can be based

The curve that has to be rectified is surrounded by another closed convex curve (circle, polygon) of the known length *L*, a great number s of arbitrary straight lines intersecting *L* are drawn in the plane of both curves, and those intersecting also the curve of the unknown length ℓ are counted; let their number be *m*.

The higher is s, the more accurately hold the equalities



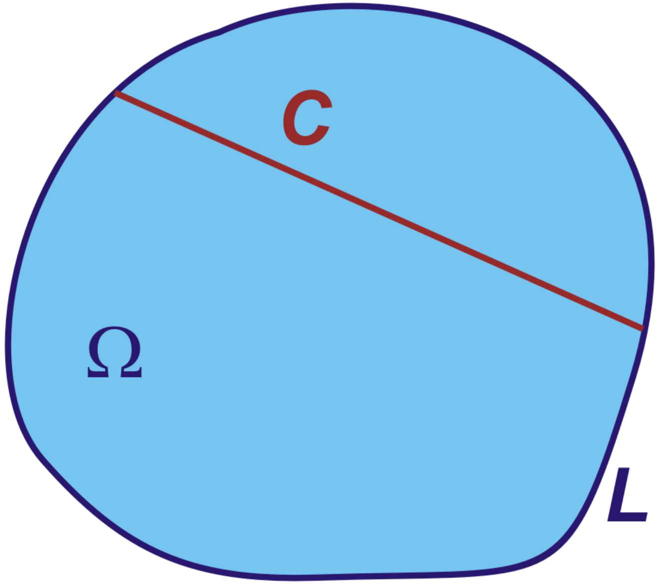
$$\frac{m}{s} = \frac{\ell}{L} \Rightarrow \ell = \frac{m}{s} L.$$

Crofton formula:

Ω ... area of a region bounded by
a closed convex curve

L ... its perimeter

C ... chord length



$$\iint C\,dp\,d\varphi = \pi\,\Omega$$

$$\iint C\,dp\,d\varphi = \frac{L}{L}\iint C\,dp\,d\varphi = L\cdot E\left(C\right) = \pi\,\Omega$$

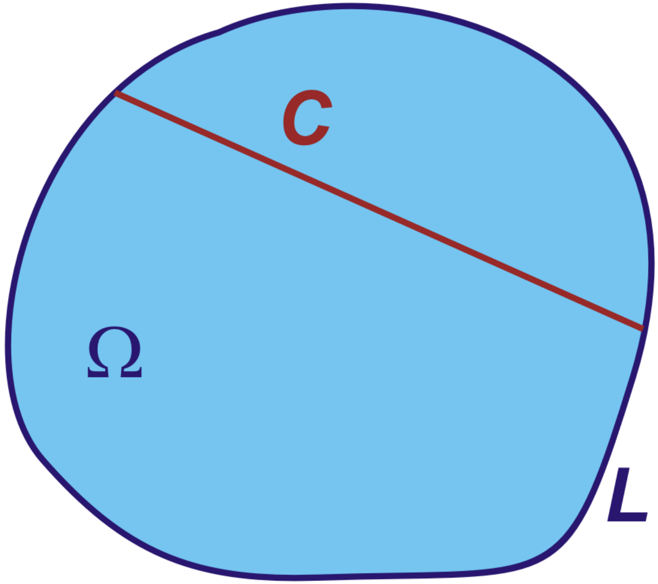
$$E(C) = \frac{\pi\,\Omega}{L}$$

Second Crofton formula:

Ω ... area of a region bounded by
a closed convex curve

L ... its perimeter

C ... chord length



$$\iint C^3 dp\,d\varphi = 3\Omega^2$$

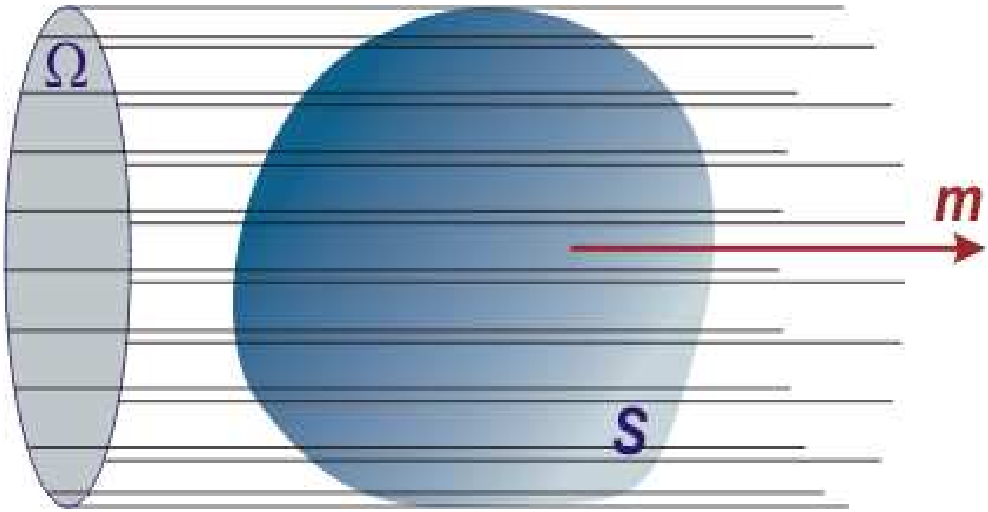
$$\iint C^3 dp\,d\varphi = \frac{L}{L}\iint C^3 dp\,d\varphi = LE(C^3) = 3\Omega^2$$

$$\Omega^2 = \frac{1}{3} LE(C^3)$$

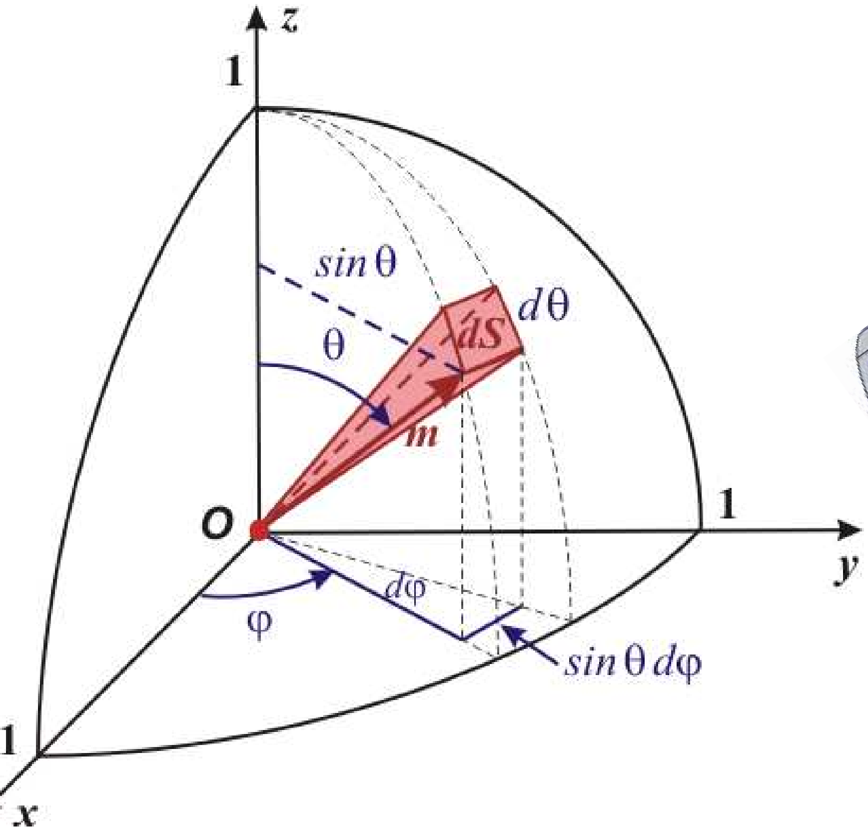
Lines in space

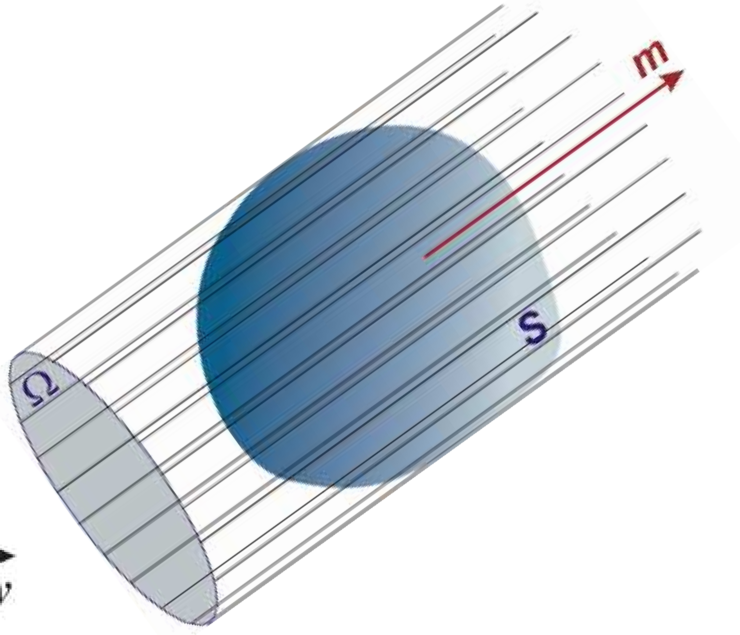
Theorem: measure of all lines hitting a closed convex surface is proportional by $\pi/2$ to its surface area

Crofton, 1868 (concluding remark), Czuber, 1884



Ω ... measure of lines hitting *S* parallel to *m*



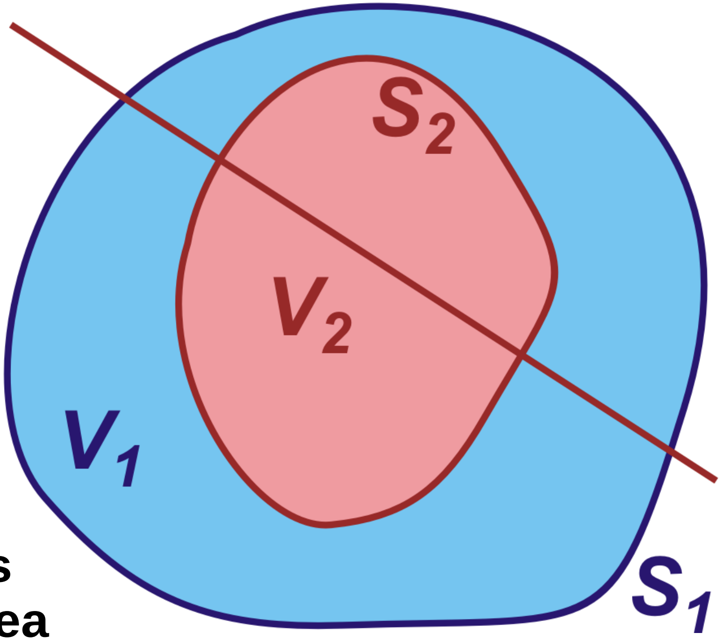


Measure of all lines hitting a surface *S*:

$$M = \int_0^{\pi}\int_0^{\pi} \Omega \sin\theta d\theta d\varphi = \frac{\pi}{2} S$$

Corollary: Probability p that a line hitting a convex surface K_1 with the surface area S_1, hits also another convex surface K_2 lying inside K_1 and having the surface area S_2:

$$p = \frac{S_2}{S_1}.$$



Nevertheless, a similar remark concerning the application of this result for the estimation of the area of a closed convex surface is not explicitly mentioned.

Planes in the space, ...

„What is it for?“

Anatomie

Histologie

Neurofyziologie

Patologie

Dermatologie

Nefrologie

Onkologie

Kardiologie

Biologie

Metalurgie

Geologie

Petrologie

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